

We Claim:

1. In a method to modulate exogenous gene expression comprising contacting an ecdysone receptor complex comprising:

- 5 a) a DNA binding domain;
- b) a ligand binding domain;
- c) a transactivation domain; and
- d) a ligand;

with a DNA construct comprising:

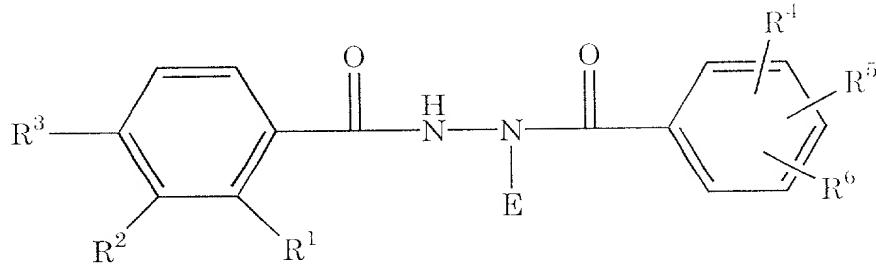
- 10 a) the exogenous gene; and
- b) a response element;

wherein:

- a) the exogenous gene is under the control of the response element; and
- b) binding of the DNA binding domain to the response element in the presence of the ligand results in activation or suppression of the gene;

the improvement comprising:

selecting the ligand from a compound of the formula:



wherein:

E is a (C<sub>4</sub>-C<sub>6</sub>)alkyl containing a tertiary carbon or a cyano(C<sub>3</sub>-C<sub>5</sub>)alkyl

20 containing a tertiary carbon;

R<sup>1</sup> is H, Me, Et, i-Pr, F, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe,

OEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, SCN, or SCHF<sub>2</sub>;

R<sup>2</sup> is H, Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH,

25 CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH,

OMe, OEt, O-n-Pr, OAc, NMe<sub>2</sub>, NET<sub>2</sub>, SMe, SET, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H,

COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>, OCHF<sub>2</sub>, O-i-Pr,

SCN, SCHF<sub>2</sub>, SOMe, NH-CN, or joined with R<sup>1</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrosuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

5 R<sup>3</sup> is H, Et, or joined with R<sup>2</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrosuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

10 R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> are independently H, Me, Et, F, Cl, Br, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SET;

provided that:

a) when R<sup>1</sup> is Me and R<sup>2</sup> is OMe;

then R<sup>3</sup> is H; and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me, 3,5-di-OMe-4-Me, 3,5-di-Cl, or 3,5-di-F;

b) when R<sup>1</sup> is Me and R<sup>2</sup> is OEt;

then R<sup>3</sup> is H and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me, 3,5-di-OMe-4-Me, 3,5-di-Cl, 3,5-di-F, 2,4- or 2,5-di-F, 2,4- or 2,5-di-Cl;

c) when R<sup>1</sup> is Et and R<sup>2</sup> is OMe or OEt;

then R<sup>3</sup> is H and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is:

i) 3,5-di-OMe-4-Me, 3,5-di-Cl, 3,5-di-F, 2,4- or 2,5-di-F, 2,4- or 2,5-di-Cl, 3-OMe, 2-Cl-5-Me, 2-Br-5-Me, 2-Cl, 2-Br, or 3-Me; or

ii) R<sup>6</sup> is H, R<sup>1</sup> is Me, and R<sup>5</sup> is Et, F, Cl, Br, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SET;

d) when R<sup>1</sup> is 1-Pr;

then R<sup>2</sup> is OMe, or OEt; R<sup>3</sup> is H; and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

e) when R<sup>3</sup> is Et;

then R<sup>2</sup> is H, R<sup>1</sup> is F or Cl, and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

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f) when R<sup>2</sup> and R<sup>3</sup>, together with the phenyl carbons to which they are attached, form an ethylenedioxy ring;  
 then R<sup>1</sup> is Me or Et and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

g) when R<sup>2</sup> and R<sup>3</sup>, together with the phenyl carbons to which they are attached, form a dihydrofuryl or dihydropyrryl ring;  
 then R<sup>1</sup> is Et and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

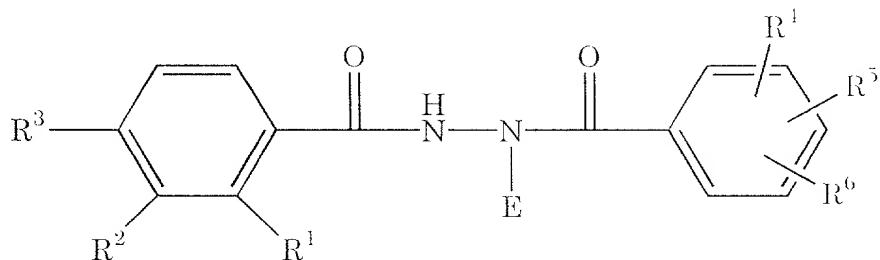
5 h) when R<sup>1</sup> is formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OH, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, SCN, or SCHF<sub>2</sub>;  
 10 then R<sup>2</sup> is OMe or OEt, R<sup>3</sup> is H, and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me; and

i) when R<sup>2</sup> is Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, O-n-Pr, OAc, NMe<sub>2</sub>, NEt<sub>2</sub>, SMe, SET, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H, COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>, OCHF<sub>2</sub>, O-i-Pr, SCN, SCHF<sub>2</sub>, SOMe, or NH-CN;  
 then R<sup>1</sup> is Et, R<sup>3</sup> is H, and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me.

2. A method to modulate exogenous gene expression comprising contacting an

20 ecdysone receptor complex comprising:

a) a DNA binding domain;  
 b) a ligand binding domain;  
 c) a transactivation domain; and  
 d) a ligand of the formula:



E is a (C<sub>1</sub>-C<sub>6</sub>)alkyl containing a tertiary carbon or a cyano(C<sub>3</sub>-C<sub>5</sub>)alkyl containing a tertiary carbon;

5 R<sup>1</sup> is H, Me, Et, i-Pr, F, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, SCN, or SCHF<sub>2</sub>;

10 R<sup>2</sup> is H, Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe<sub>2</sub>, NEt<sub>2</sub>, SMe, SET, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H, COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>, OCHF<sub>2</sub>, O-i-Pr, SCN, SCHF<sub>2</sub>, SOMe, NH-CN, or joined with R<sup>3</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrosuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

15 R<sup>3</sup> is H, Et, or joined with R<sup>2</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrosuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

20 R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> are independently H, Me, Et, F, Cl, Br, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SET;

provided that:

a) when R<sup>1</sup> is Me and R<sup>2</sup> is OMe;

25 then R<sup>3</sup> is H; and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me, 3,5-di-OMe-4-Me, 3,5-di-Cl, or 3,5-di-F;

b) when R<sup>1</sup> is Me and R<sup>2</sup> is OEt;

then R<sup>3</sup> is H and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me, 3,5-di-OMe-4-Me, 3,5-di-Cl, 3,5-di-F, 2,4- or 2,5-di-F, 2,4- or 2,5-di-Cl;

c) when R<sup>1</sup> is Et and R<sup>2</sup> is OMe or OEt;

30 then R<sup>3</sup> is H and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is:

1) 3,5-di-OMe-4-Me, 3,5-di-Cl, 3,5-di-F, 2,4- or 2,5-di-F, 2,4- or 2,5-di-Cl, 3-OMe, 2-Cl-5-Me, 2-Br-5-Me, 2-Cl, 2-Br, or 3-Me; or

5 ii) R<sup>6</sup> is H, R<sup>4</sup> is Me, and R<sup>5</sup> is Et, F, Cl, Br, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SET;

d) when R<sup>1</sup> is i-Pr;

10 then R<sup>2</sup> is OMe, or OEt; R<sup>3</sup> is H; and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

e) when R<sup>3</sup> is Et;

15 then R<sup>2</sup> is H, R<sup>1</sup> is F or Cl, and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

f) when R<sup>2</sup> and R<sup>3</sup>, together with the phenyl carbons to which they are attached, form an ethylenedioxy ring;

then R<sup>1</sup> is Me or Et and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

20 g) when R<sup>2</sup> and R<sup>3</sup>, together with the phenyl carbons to which they are attached, form a dihydrofuryl or dihydropyryl ring,

then R<sup>1</sup> is Et and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

h) when R<sup>1</sup> is formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OH, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, SCN, or SCHF<sub>2</sub>;

25 then R<sup>2</sup> is OMe or OEt, R<sup>3</sup> is H, and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me; and

i) when R<sup>2</sup> is Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, O-n-Pr, OAc, NMe<sub>2</sub>, NEt<sub>2</sub>, SMe, SET, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H, COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>, OCHF<sub>2</sub>, O-i-Pr, SCN, SCHF<sub>2</sub>, SMe, or NH-CN;

then R<sup>1</sup> is Et, R<sup>3</sup> is H, the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

with a DNA construct comprising:

30 a) the exogenous gene; and

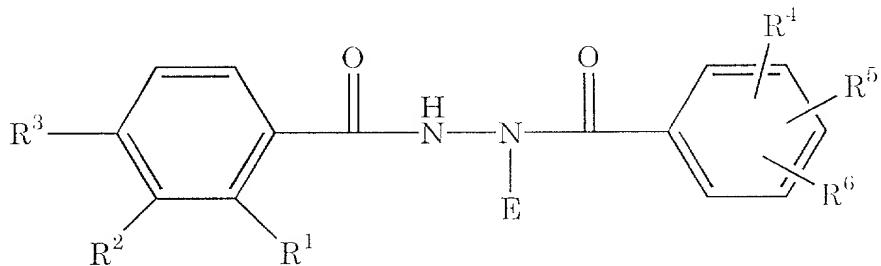
b) a response element;

wherein:

- a) the exogenous gene is under the control of the response element; and
- b) binding of the DNA binding domain to the response element in the presence of the ligand results in activation or suppression of the gene.

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3. A method to modulate the expression of one or more exogenous genes in a subject, comprising administering to the subject an effective amount of a ligand of the formula:



wherein:

E is a (C<sub>4</sub>-C<sub>6</sub>)alkyl containing a tertiary carbon or a cyano(C<sub>3</sub>-C<sub>5</sub>)alkyl containing a tertiary carbon;

R<sup>1</sup> is H, Me, Et, i-Pr, F, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, SCN, or SCHF<sub>2</sub>;

R<sup>2</sup> is H, Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe<sub>2</sub>, NEt<sub>2</sub>, SMe, SET, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H, COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>, OCHF<sub>2</sub>, O-i-Pr, SCN, SCHF<sub>2</sub>, SOMe, NH-CN, or joined with R<sup>3</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

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R<sup>3</sup> is H, Et, or joined with R<sup>2</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the

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oxygen adjacent to a phenyl carbon, or a dihydropyrryl ring with the oxygen adjacent to a phenyl carbon;

R<sup>1</sup>, R<sup>5</sup>, and R<sup>6</sup> are independently H, Me, Et, F, Cl, Br, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SEt;

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provided that:

a) when R<sup>1</sup> is Me and R<sup>2</sup> is OMe;

then R<sup>3</sup> is H; and the combination R<sup>1</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me, 3,5-di-OMe-4-Me, 3,5-di-Cl, or 3,5-di-F;

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b) when R<sup>1</sup> is Me and R<sup>2</sup> is OEt;

then R<sup>3</sup> is H and the combination R<sup>1</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me, 3,5-di-OMe-4-Me, 3,5-di-Cl, 3,5-di-F, 2,4- or 2,5-di-F, 2,4- or 2,5-di-Cl;

c) when R<sup>1</sup> is Et and R<sup>2</sup> is OMe or OEt;

then R<sup>3</sup> is H and the combination R<sup>1</sup>, R<sup>5</sup>, and R<sup>6</sup> is:

- i) 3,5-di-OMe-4-Me, 3,5-di-Cl, 3,5-di-F, 2,4- or 2,5-di-F, 2,4- or 2,5-di-Cl, 3-OMe, 2-Cl-5-Me, 2-Br-5-Me, 2-Cl, 2-Br, or 3-Me; or
- ii) R<sup>6</sup> is H, R<sup>4</sup> is Me, and R<sup>5</sup> is Et, F, Cl, Br, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SEt;

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d) when R<sup>1</sup> is i-Pr;

then R<sup>2</sup> is OMe, or OEt; R<sup>3</sup> is H; and the combination R<sup>1</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

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e) when R<sup>3</sup> is Et;

then R<sup>2</sup> is H, R<sup>1</sup> is F or Cl, and the combination R<sup>1</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

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f) when R<sup>2</sup> and R<sup>3</sup>, together with the phenyl carbons to which they are attached, form an ethylenedioxy ring;

then R<sup>1</sup> is Me or Et and the combination R<sup>1</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

g) when R<sup>2</sup> and R<sup>3</sup>, together with the phenyl carbons to which they are attached, form a dihydrosaryl or dihydropyrryl ring;

then R<sup>1</sup> is Et and the combination R<sup>1</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

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h) when R<sup>1</sup> is formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OH, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, SCN, or SCHF<sub>2</sub>;  
then R<sup>2</sup> is OMe or OEt, R<sup>3</sup> is H, and the combination R<sup>1</sup>, R<sup>5</sup>, and R<sup>6</sup> is  
5 3,5-di-Me; and

1) when R<sup>2</sup> is Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, O-n-Pr, OAc, NMe<sub>2</sub>, NEt<sub>2</sub>, SMe, SEt, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H, COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>, OCHF<sub>2</sub>, O-  
10 i-Pr, SCN, SCHF<sub>2</sub>, SOMe, or NH-CN;  
then R<sup>1</sup> is Et, R<sup>3</sup> is H, the combination R<sup>1</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

wherein the cells of the subject contain:

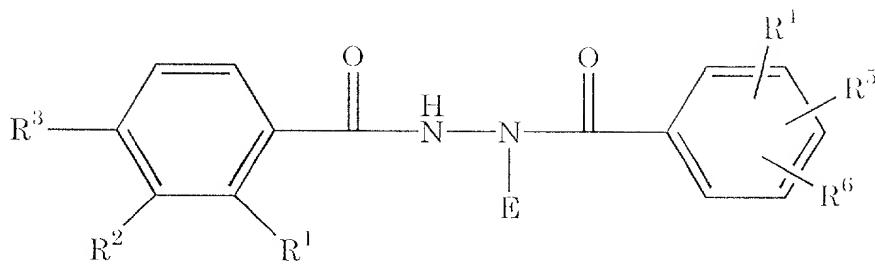
- a) an ecdysone receptor complex comprising:
  - 1) a DNA binding domain;
  - 2) a binding domain for the ligand; and
  - 3) a transactivation domain; and
- b) a DNA construct comprising:
  - 1) the exogenous gene; and
  - 2) a response element; and

20 wherein:

- a) the exogenous gene is under the control of the response element; and
- b) binding of the DNA binding domain to the response element in the presence of the ligand results in activation or suppression of the gene.

25 4. A method for producing a polypeptide comprising the steps of:

- a) selecting a cell which is substantially insensitive to exposure to a ligand of the formula:



wherein:

E is a (C<sub>4</sub>-C<sub>6</sub>)alkyl containing a tertiary carbon or a cyano(C<sub>3</sub>-C<sub>5</sub>)alkyl containing a tertiary carbon;

5 R<sup>1</sup> is H, Me, Et, i-Pr, F, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, SCN, or SCHF<sub>2</sub>;

R<sup>2</sup> is H, Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe<sub>2</sub>, NEt<sub>2</sub>, SMe, SET, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H, COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>, OCHF<sub>2</sub>, O-1-Pr, SCN, SCHF<sub>2</sub>, SOMe, NH-CN, or joined with R<sup>3</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

20 R<sup>3</sup> is H, Et, or joined with R<sup>2</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> are independently H, Me, Et, F, Cl, Br, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SET;

provided that:

25 a) when R<sup>1</sup> is Me and R<sup>2</sup> is OMe;

then R<sup>3</sup> is H; and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me, 3,5-di-OMe-4-Me, 3,5-di-Cl, or 3,5-di-F;

b) when R<sup>1</sup> is Me and R<sup>2</sup> is OEt;

then R<sup>3</sup> is H and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me, 3,5-di-OMe-4-Me, 3,5-di-Cl, 3,5-di-F, 2,4- or 2,5-di-F, 2,4- or 2,5-di-Cl;

c) when R<sup>1</sup> is Et and R<sup>2</sup> is OMe or OEt;

then R<sup>3</sup> is H and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is:

i) 3,5-di-OMe-4-Me, 3,5-di-Cl, 3,5-di-F, 2,4- or 2,5-di-F, 2,4- or 2,5-di-Cl, 3-OMe, 2-Cl-5-Me, 2-Br-5-Me, 2-Cl, 2-Br, or 3-Me; or

ii) R<sup>6</sup> is H, R<sup>4</sup> is Me, and R<sup>5</sup> is Et, F, Cl, Br, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl,

OMe, OEt, SMe, or SEt;

d) when R<sup>1</sup> is i-Pr;

then R<sup>2</sup> is OMe, or OEt; R<sup>3</sup> is H; and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

e) when R<sup>3</sup> is Et;

then R<sup>2</sup> is H, R<sup>1</sup> is F or Cl, and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

f) when R<sup>2</sup> and R<sup>3</sup>, together with the phenyl carbons to which they are attached, form an ethylenedioxy ring;

then R<sup>1</sup> is Me or Et and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

g) when R<sup>2</sup> and R<sup>3</sup>, together with the phenyl carbons to which they are attached, form a dihydrofuryl or dihydropyryl ring;

then R<sup>1</sup> is Et and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

h) when R<sup>1</sup> is formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OH, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, SCN, or SCHF<sub>2</sub>:

then R<sup>2</sup> is OMe or OEt, R<sup>3</sup> is H, and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me; and

i) when R<sup>2</sup> is Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, O-n-Pr, OAc, NMe<sub>2</sub>, NEt<sub>2</sub>, SMe, SEt, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H,

COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>, OCHF<sub>2</sub>, O-i-Pr, SCN, SCHF<sub>2</sub>, SOMe, or NH-CN;

then R<sup>1</sup> is Et, R<sup>3</sup> is H, the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

b) introducing into the cell:

5 1) a DNA construct comprising:

- a) an exogenous gene encoding the polypeptide; and
- b) a response element;

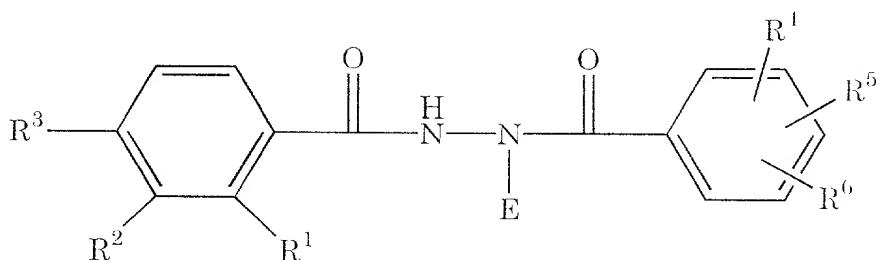
wherein the gene is under the control of the response element; and

10 2) an ecdysone receptor complex comprising:

- a) a DNA binding domain;
- b) a binding domain for the ligand; and
- c) a transactivation domain; and

c) exposing the cell to the ligand.

5. A method for regulating endogenous or heterologous gene expression in a transgenic organism comprising contacting a ligand of the formula:



wherein:

E is a (C<sub>4</sub>-C<sub>6</sub>)alkyl containing a tertiary carbon or a cyano(C<sub>3</sub>-C<sub>5</sub>)alkyl containing a tertiary carbon;

R<sup>1</sup> is H, Me, Et, i-Pr, F, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, SCN, or SCHF<sub>2</sub>;

R<sup>2</sup> is H, Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe<sub>2</sub>, NEt<sub>2</sub>, SMe, SEt, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H, COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>, OCHF<sub>2</sub>, O-i-Pr, SCN, SCHF<sub>2</sub>, SOMe, NH-CN, or joined with R<sup>3</sup> and the

phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

5 R<sup>3</sup> is H, Et, or joined with R<sup>2</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

10 R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> are independently H, Me, Et, F, Cl, Br, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SET;

provided that:

a) when R<sup>1</sup> is Me and R<sup>2</sup> is OMe;

then R<sup>3</sup> is H; and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me, 3,5-di-OMe-4-Me, 3,5-di-Cl, or 3,5-di-F;

b) when R<sup>1</sup> is Me and R<sup>2</sup> is OEt;

then R<sup>3</sup> is H and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me, 3,5-di-OMe-4-Me, 3,5-di-Cl, 3,5-di-F, 2,4- or 2,5-di-F, 2,4- or 2,5-di-Cl;

c) when R<sup>1</sup> is Et and R<sup>2</sup> is OMe or OEt;

then R<sup>3</sup> is H and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is:

i) 3,5-di-OMe-4-Me, 3,5-di-Cl, 3,5-di-F, 2,4- or 2,5-di-F, 2,4- or 2,5-di-Cl, 3-OMe, 2-Cl-5-Me, 2-Br-5-Me, 2-Cl, 2-Br, or 3-Me; or

ii) R<sup>6</sup> is H, R<sup>4</sup> is Me, and R<sup>5</sup> is Et, F, Cl, Br, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SET;

d) when R<sup>1</sup> is i-Pr;

then R<sup>2</sup> is OMe, or OEt; R<sup>3</sup> is H; and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

e) when R<sup>3</sup> is Et;

then R<sup>2</sup> is H, R<sup>1</sup> is F or Cl, and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

5 f) when R<sup>2</sup> and R<sup>3</sup>, together with the phenyl carbons to which they are attached, form an ethylenedioxy ring;  
 then R<sup>1</sup> is Me or Et and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

g) when R<sup>2</sup> and R<sup>3</sup>, together with the phenyl carbons to which they are attached, form a dihydrofuryl or dihydropyryl ring;  
 then R<sup>1</sup> is Et and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

10 h) when R<sup>1</sup> is formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, OH, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, SCN, or SCHF<sub>2</sub>;  
 then R<sup>2</sup> is OMe or OEt, R<sup>3</sup> is H, and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me; and

15 i) when R<sup>2</sup> is Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C≡CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, O-n-Pr, OAc, NMe<sub>2</sub>, NET<sub>2</sub>, SMe, SET, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H, COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>, OCHF<sub>2</sub>, O-i-Pr, SCN, SCHF<sub>2</sub>, SOME, or NH-CN;  
 then R<sup>1</sup> is Et, R<sup>3</sup> is H, the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;  
 with an ecdysone receptor complex within the cells of the organism wherein  
 the cells further contain a DNA binding sequence for the ecdysone  
 receptor complex when in combination with the ligand and wherein  
 formation of an ecdysone receptor complex-ligand-DNA binding sequence  
 complex induces expression of the gene.

20 6. The method of Claim 2 wherein the ligand is of the specified formula and E is  
 t-butyl; R<sup>1</sup> is Me, Et, i-Pr, or F; R<sup>2</sup> is OH, OMe, OEt, or joined with R<sup>3</sup> and the  
 phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy or  
 dihydrofuryl ring with the oxygen adjacent to a phenyl carbon; R<sup>3</sup> is H, Et or  
 joined with R<sup>2</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to  
 form an ethylenedioxy or dihydrofuryl ring with the oxygen adjacent to a  
 phenyl carbon; and R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> are independently Me, F, Cl, CH<sub>2</sub>OH, or  
 25 OMe.

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7. The method of Claim 3 wherein the ligand is of the specified formula and E is t-butyl; R<sup>1</sup> is Me, Et, i-Pr, or F; R<sup>2</sup> is OH, OMe, OEt, or joined with R<sup>3</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy or dihydrofuryl ring with the oxygen adjacent to a phenyl carbon; R<sup>3</sup> is H, Et or joined with R<sup>2</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy or dihydrofuryl ring with the oxygen adjacent to a phenyl carbon; and R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> are independently Me, F, Cl, CH<sub>2</sub>OH, or OMe.

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8. The method of Claim 4 wherein the ligand is of the specified formula and E is t-butyl; R<sup>1</sup> is Me, Et, i-Pr, or F; R<sup>2</sup> is OH, OMe, OEt, or joined with R<sup>3</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy or dihydrofuryl ring with the oxygen adjacent to a phenyl carbon; R<sup>3</sup> is H, Et or joined with R<sup>2</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy or dihydrofuryl ring with the oxygen adjacent to a phenyl carbon; and R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> are independently Me, F, Cl, CH<sub>2</sub>OH, or OMe.

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9. The method of Claim 5 wherein the ligand is of the specified formula and E is t-butyl; R<sup>1</sup> is Me, Et, i-Pr, or F; R<sup>2</sup> is OH, OMe, OEt, or joined with R<sup>3</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy or dihydrofuryl ring with the oxygen adjacent to a phenyl carbon; R<sup>3</sup> is H, Et or joined with R<sup>2</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy or dihydrofuryl ring with the oxygen adjacent to a phenyl carbon; and R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> are independently Me, F, Cl, CH<sub>2</sub>OH, or OMe.

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10. The method of Claim 2 wherein the ligand is of the specified formula and E is t-butyl, R<sup>1</sup> is Et, R<sup>2</sup> is OEt, R<sup>3</sup> is H, and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me.

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11. The method of Claim 3 wherein the ligand is of the specified formula and E is t-butyl, R<sup>1</sup> is Et, R<sup>2</sup> is OEt, R<sup>3</sup> is H, and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me.

30

12. The method of Claim 4 wherein the ligand is of the specified formula and E is t-butyl, R<sup>1</sup> is Et, R<sup>2</sup> is OEt, R<sup>3</sup> is H, and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me.

13. The method of Claim 5 wherein the ligand is of the specified formula and E is t-butyl, R<sup>1</sup> is Et, R<sup>2</sup> is OEt, R<sup>3</sup> is H, and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me.

14. The method of Claim 2 wherein the ecdysone receptor complex is a chimeric ecdysone receptor complex and the DNA construct further comprises a promoter.

15. The method of Claim 3 wherein the ecdysone receptor complex is a chimeric ecdysone receptor complex and the DNA construct further comprises a promoter.

16. The method of Claim 4 wherein the ecdysone receptor complex is a chimeric ecdysone receptor complex and the DNA construct further comprises a promoter.

17. The method of Claim 5 wherein the ecdysone receptor complex is a chimeric ecdysone receptor complex and the DNA construct further comprises a promoter.

18. The method of Claim 3 wherein the subject is a plant

19. The method of Claim 3 wherein the subject is a mammal.